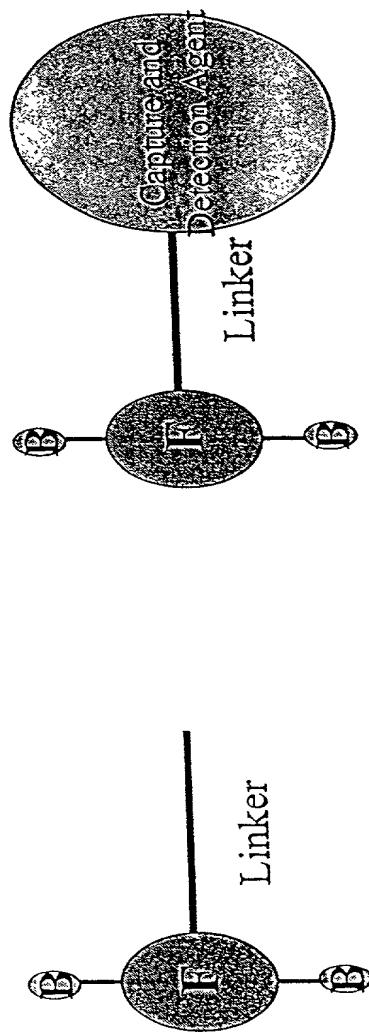


### High-throughput Target ID



### Library of Bioactive Compounds

### Library of Target ID Compounds

Use corresponding activity-based probe to identify the biological target

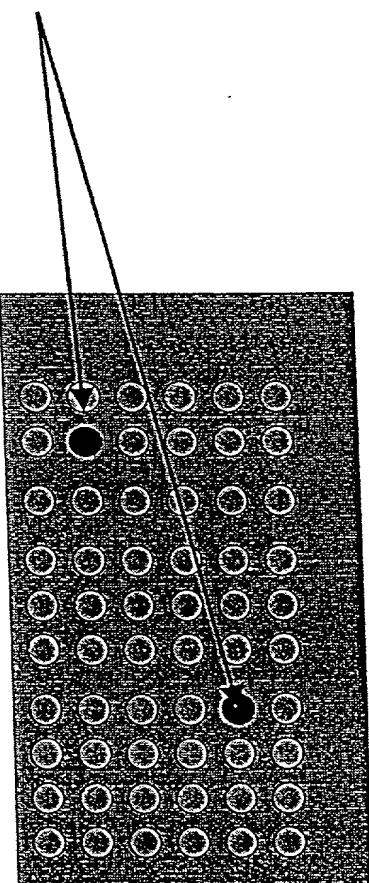
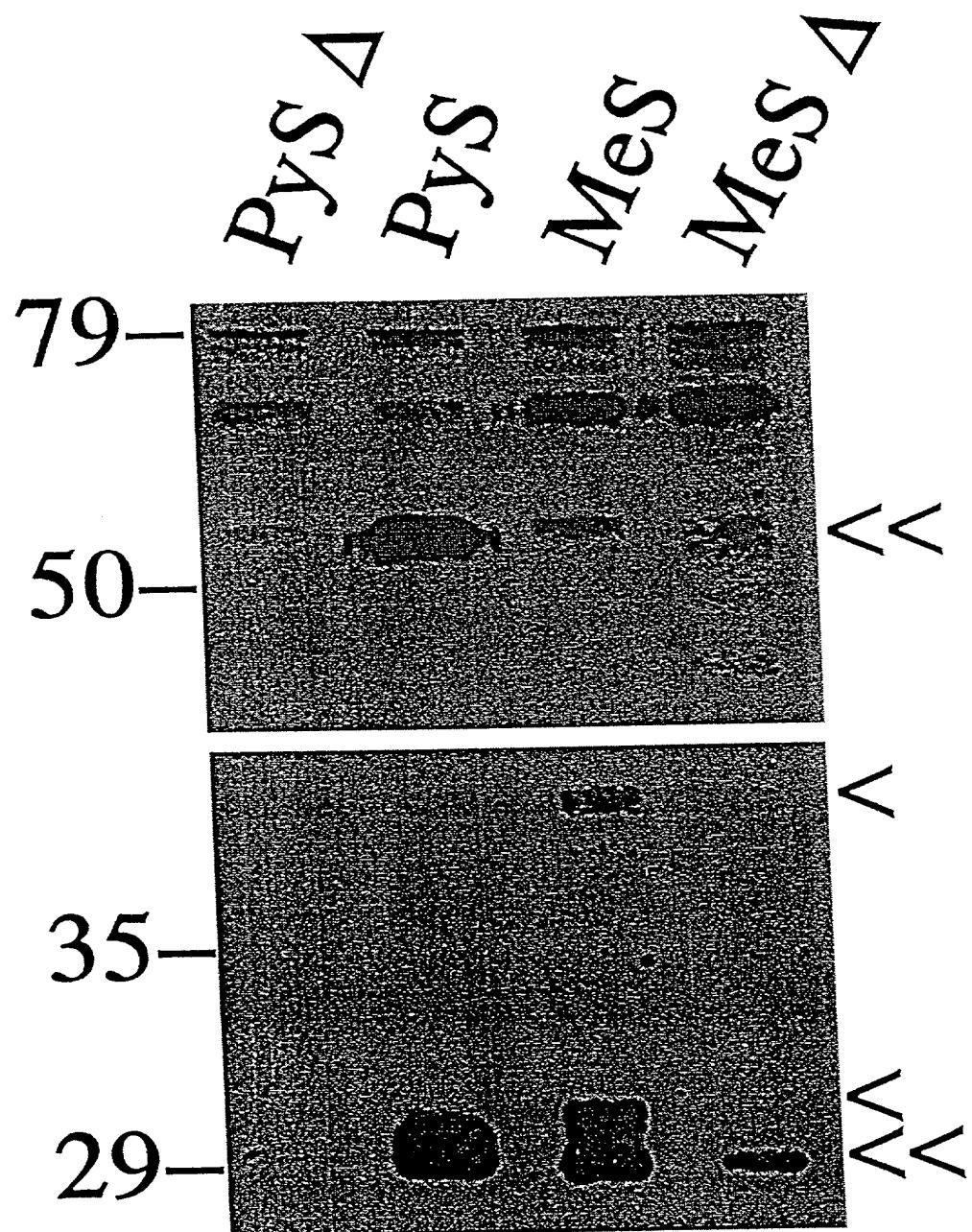


FIGURE 1

FIGURE 2



Non-Directed Tagged Library of Sulfonates Identifies Probe for  
ADH Superfamily of Enzymes

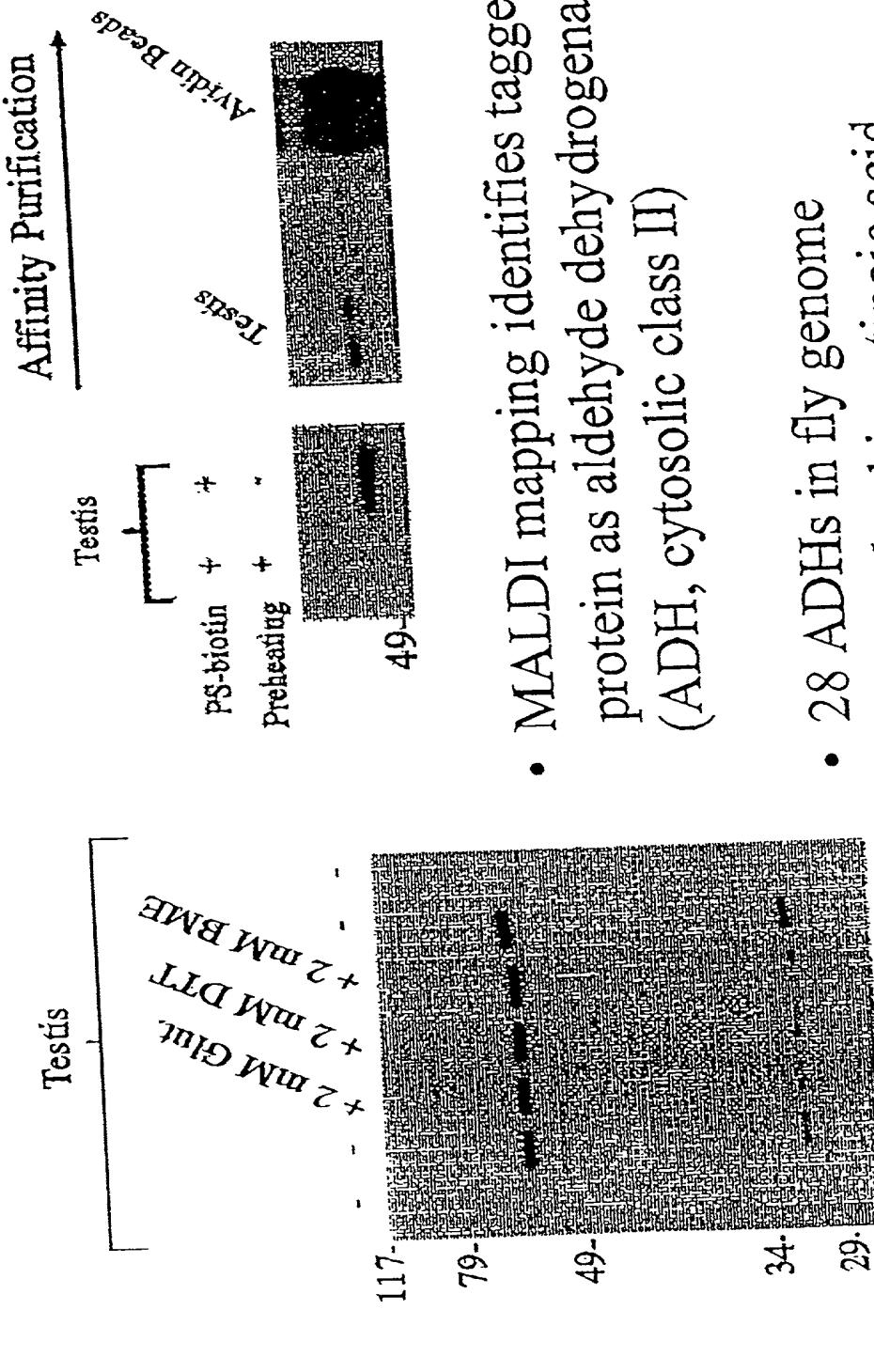
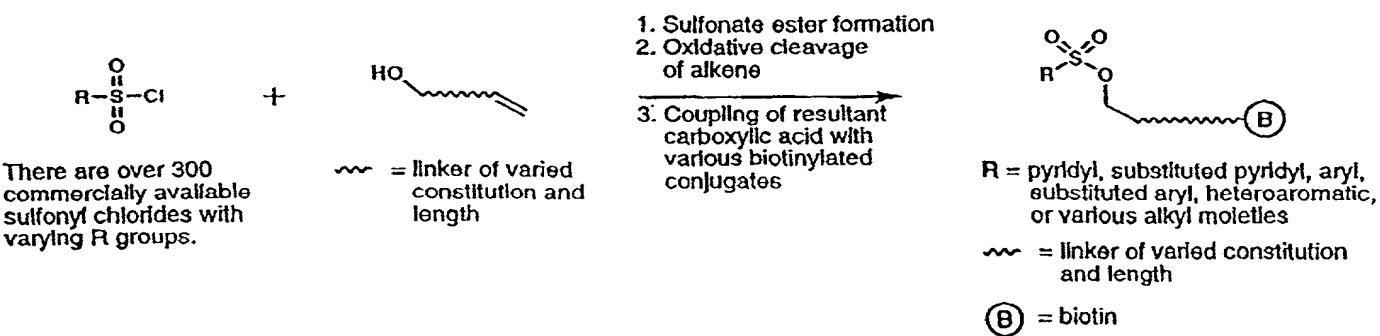


FIGURE 3

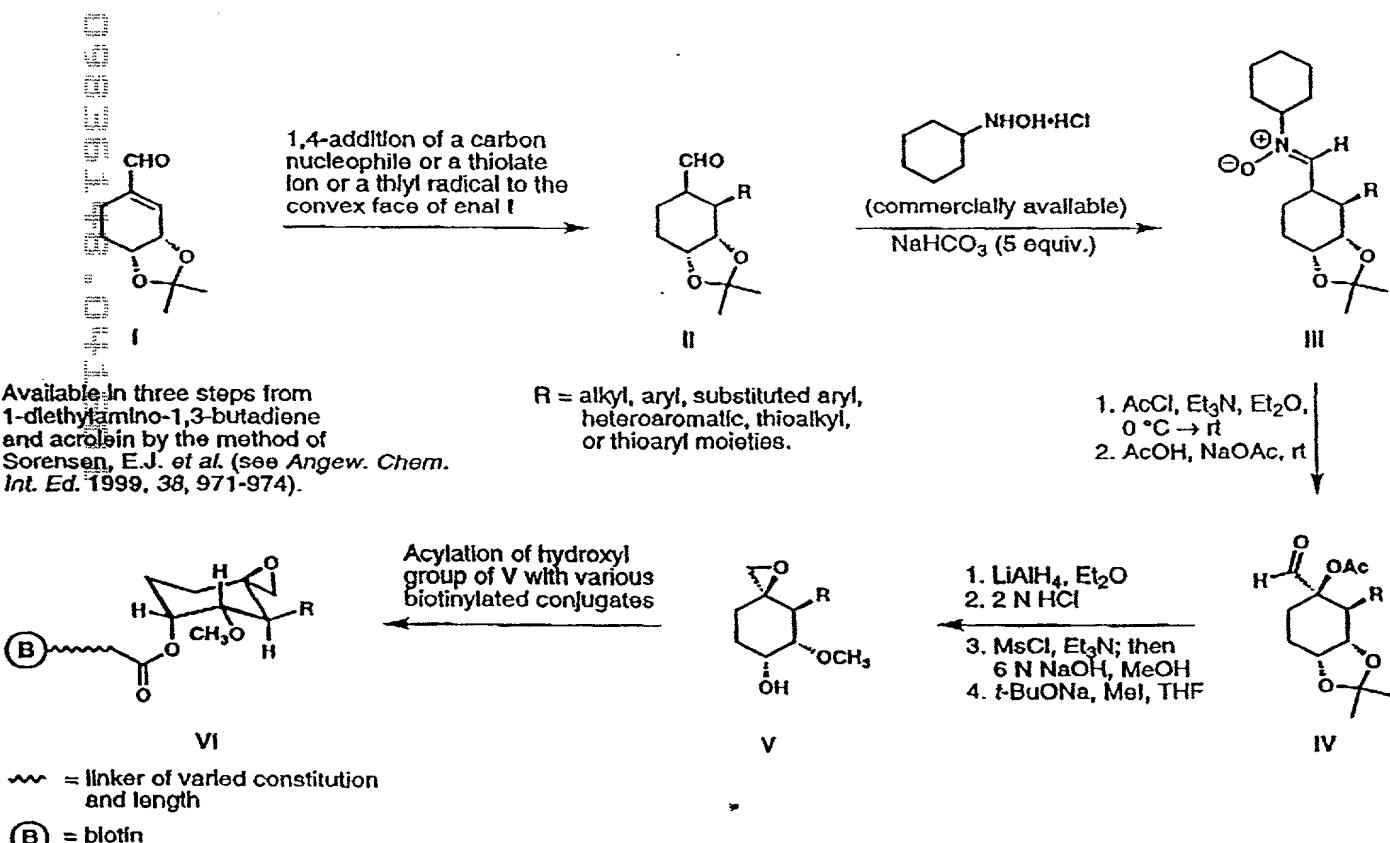
- MALDI mapping identifies tagged protein as aldehyde dehydrogenase (ADH, cytosolic class II)
- 28 ADHs in fly genome
  - Involved in retinoic acid biosynthesis and catabolism of alcohol and chemotherapeutic agents

	PS-biotin	+	+	+	+	+
29.	+	+	+	+	+	+
Preheating	+	-	-	-	-	+

FIGURE 4



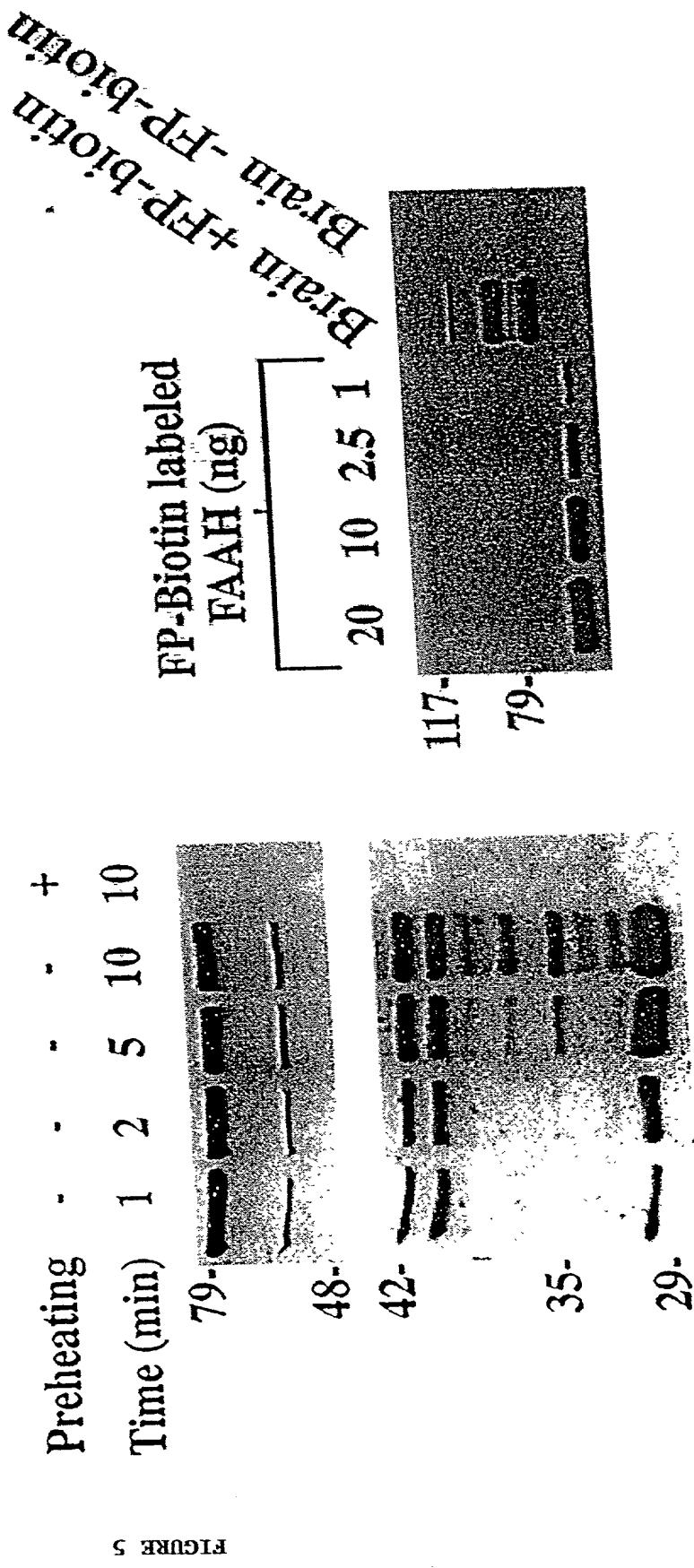
Scheme 1. A pathway for syntheses of various biotinylated sulfonate esters for use in activity-based proteomics studies.



Scheme 2. A strategy for convergent, stereocontrolled syntheses of conformationally well-defined spiroepoxides of type VI. Literature precedent for I  $\rightarrow$  II  $\rightarrow$  III  $\rightarrow$  IV  $\rightarrow$  V can be found in Sorensen, E.J. et al. *Angew. Chem. Int. Ed.* 1999, 38, 971-974. Compounds of type VI are analogs of the metalloprotease (MetAp-2) inhibitor fumagillin and will be employed as covalent affinity agents in activity-based proteomics studies.

## FP-Biotin: a kinetic reporter of SH Activity

- The rates at which the majority of SHs react with FP-biotin can be experimentally followed
  - FP-biotin readily detects low femtomole quantities of SHs directly in complex cell/tissue proteomes



# Utility of Multiplexed probes in identifying Serine Hydrolases

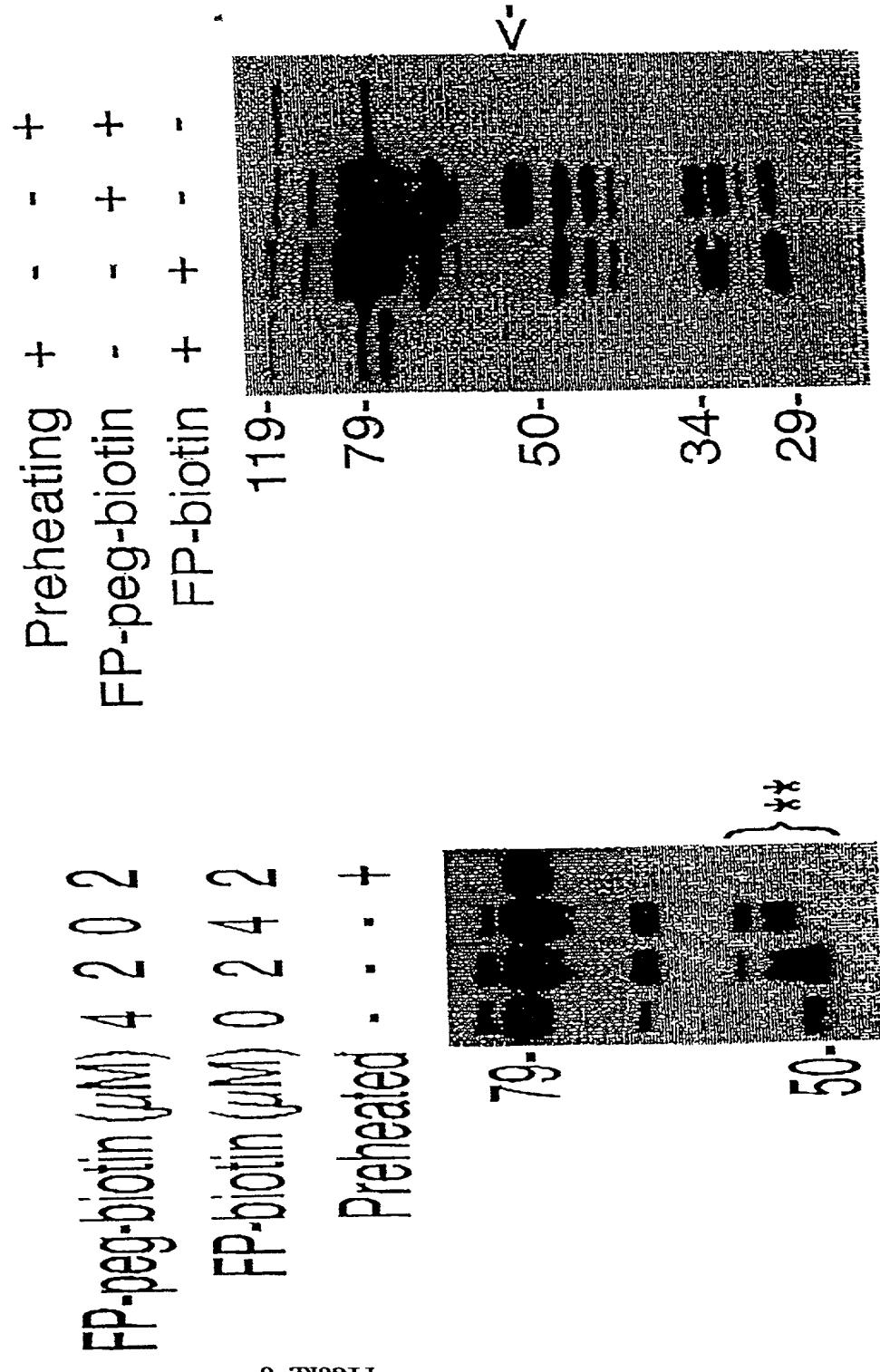


FIGURE 7

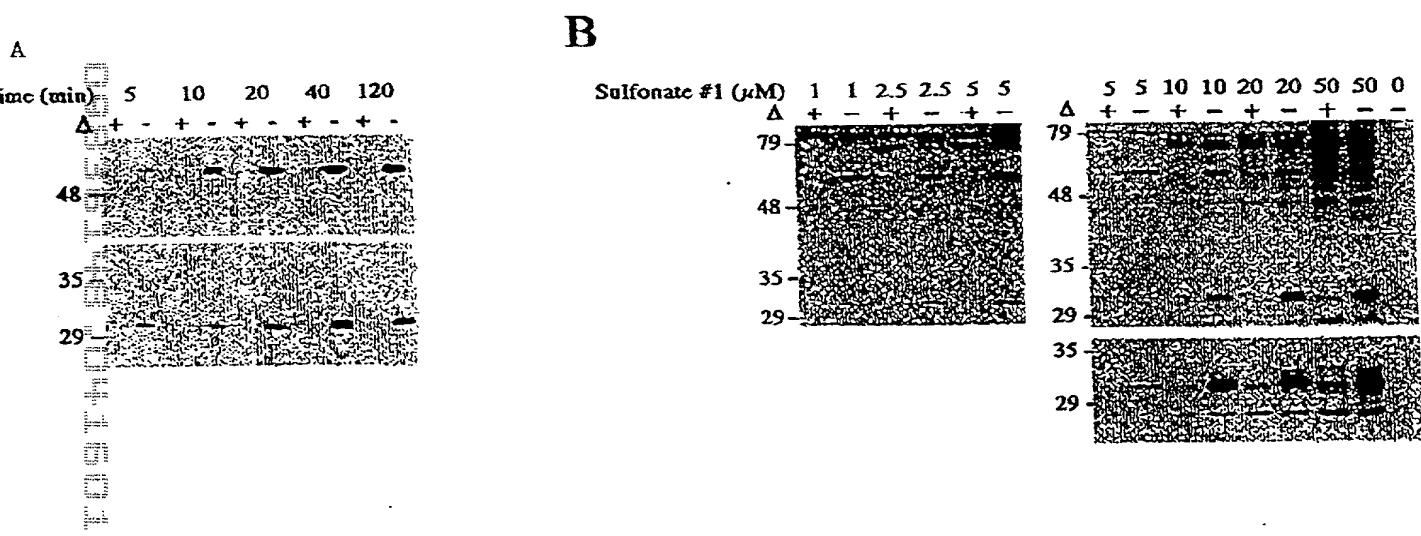
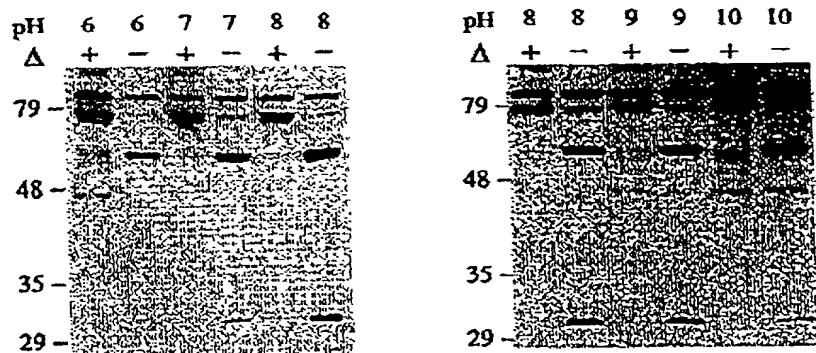


FIGURE 7

C



D

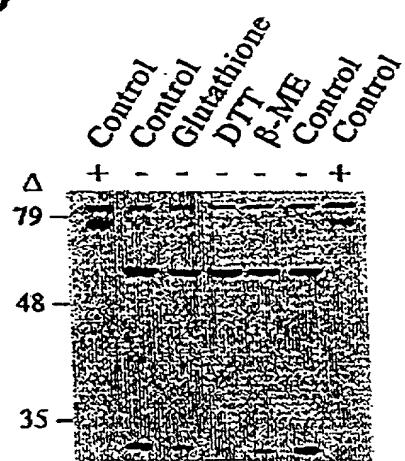


FIGURE 8

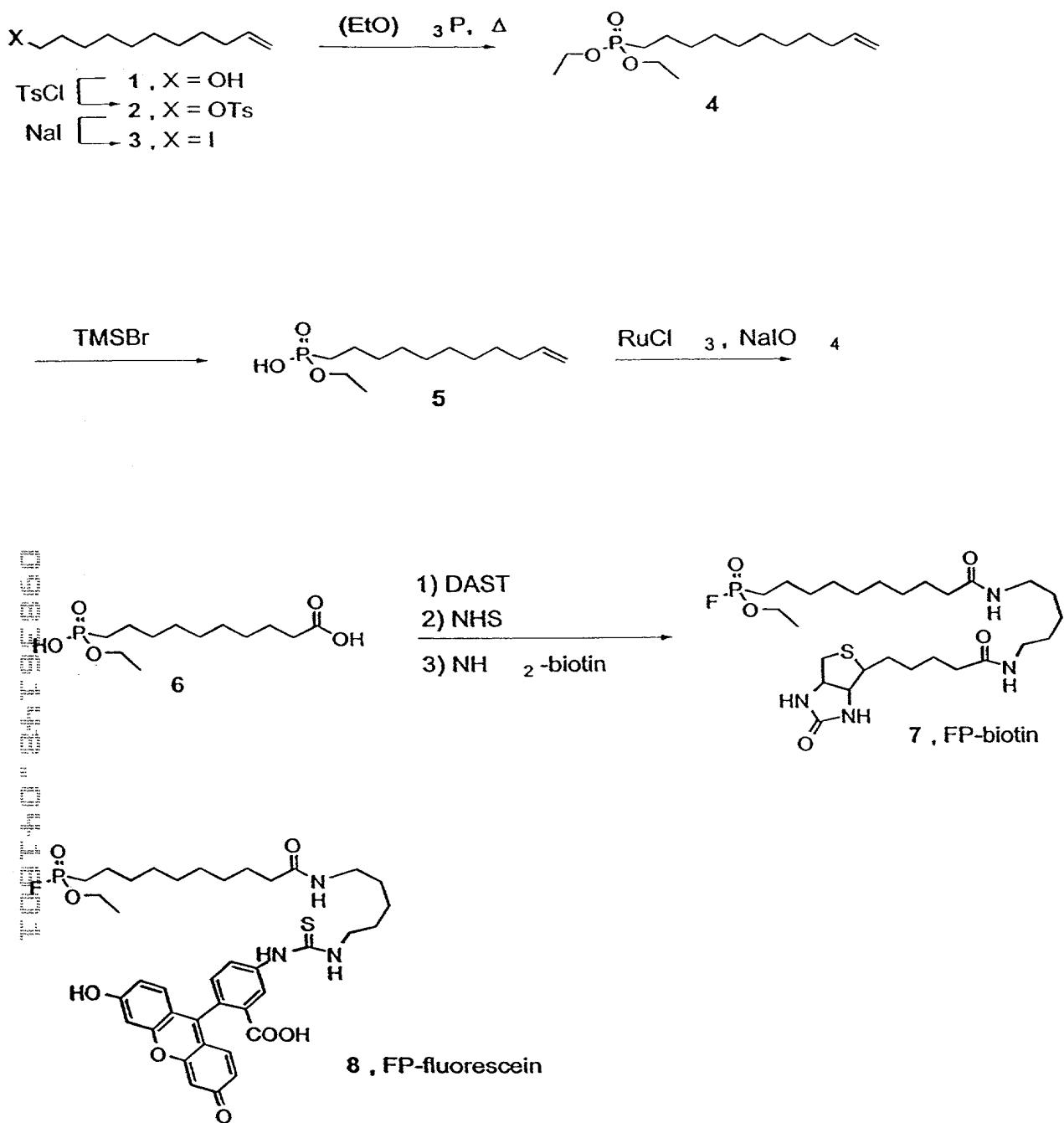


FIGURE 9

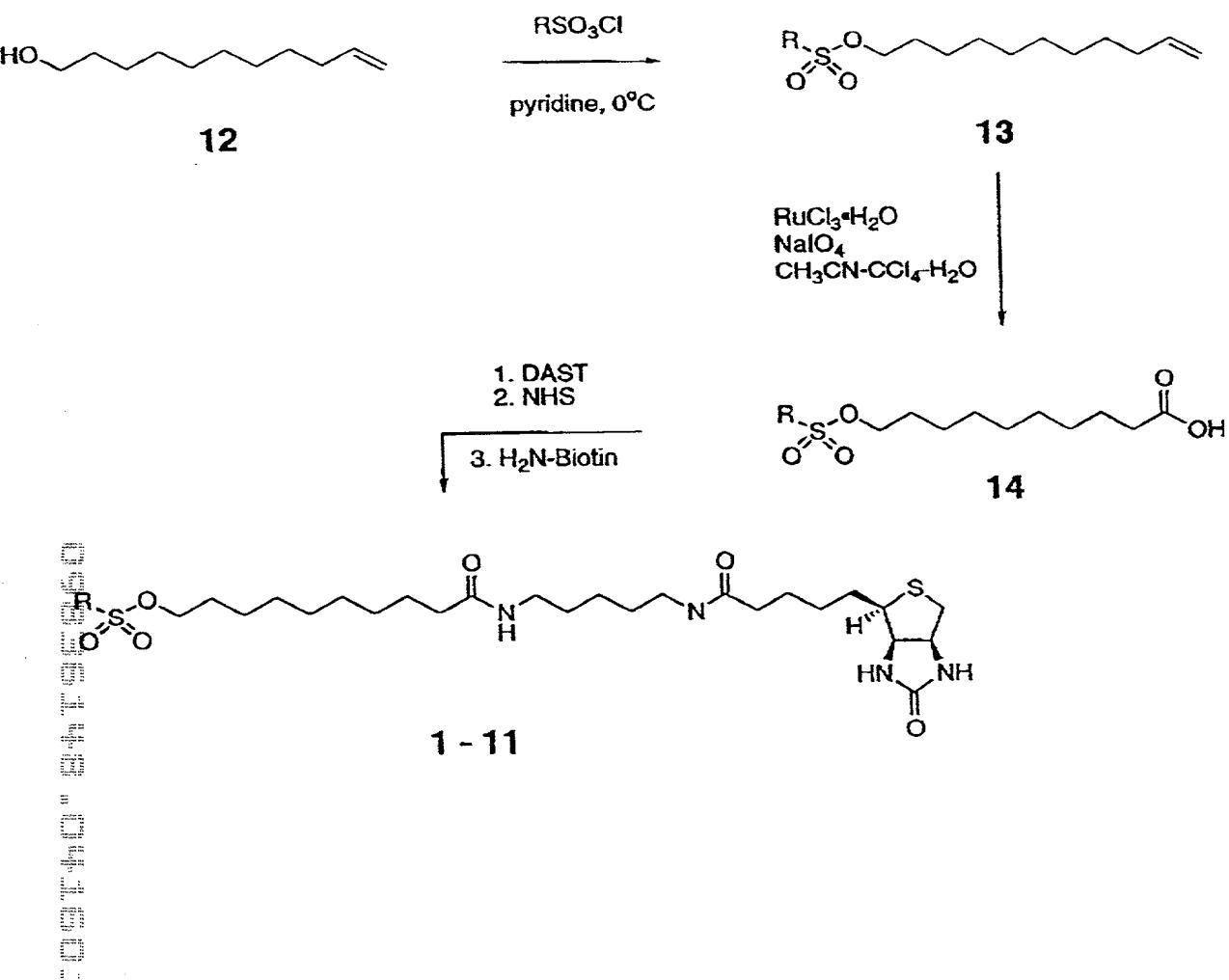
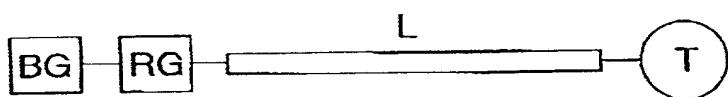


FIGURE 10

A.



B.

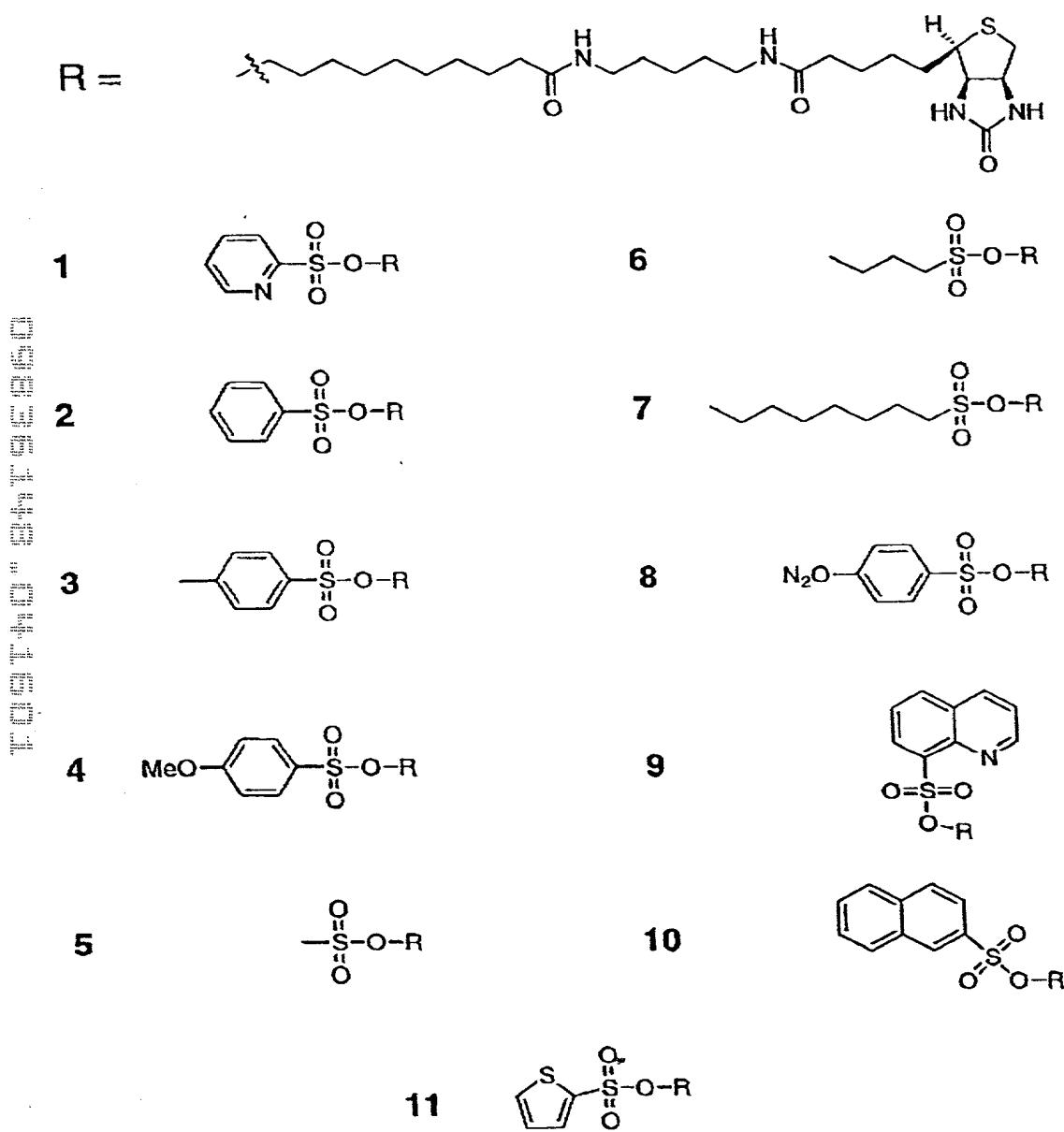
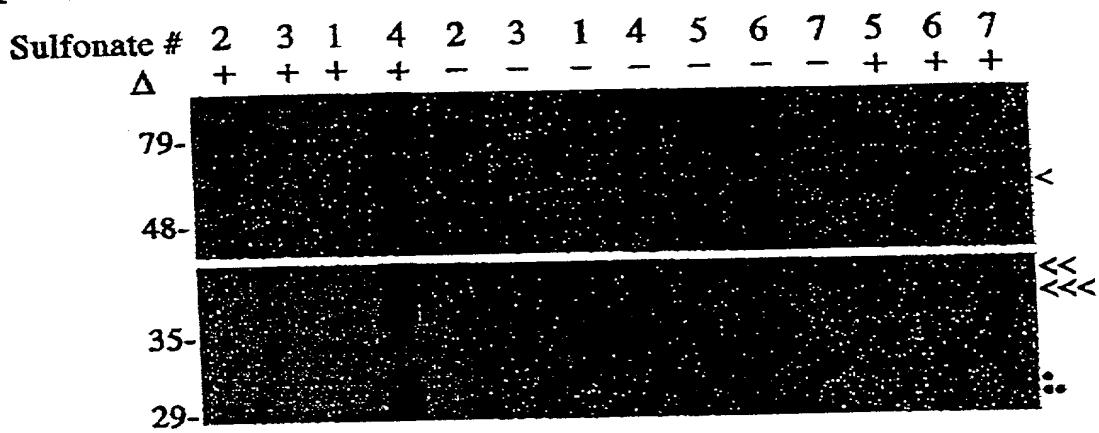


FIGURE 11

A



B

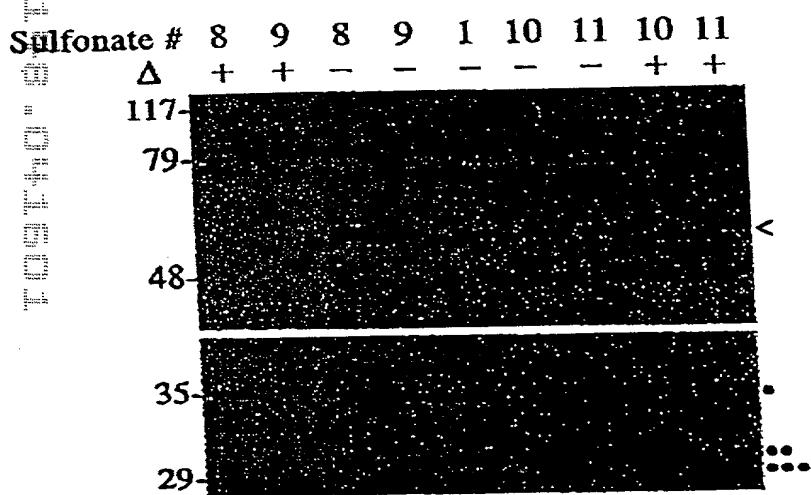


FIGURE 12

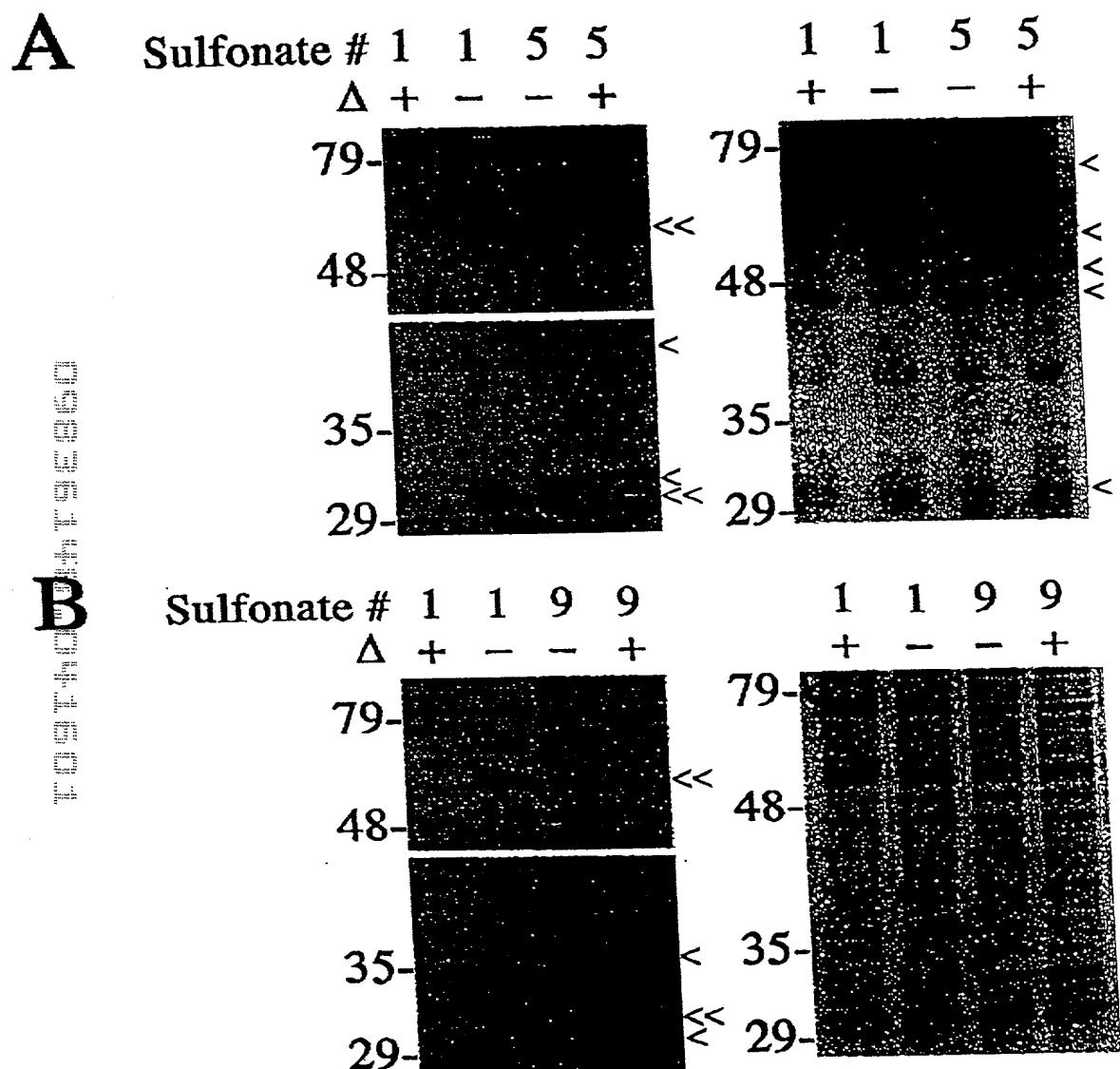
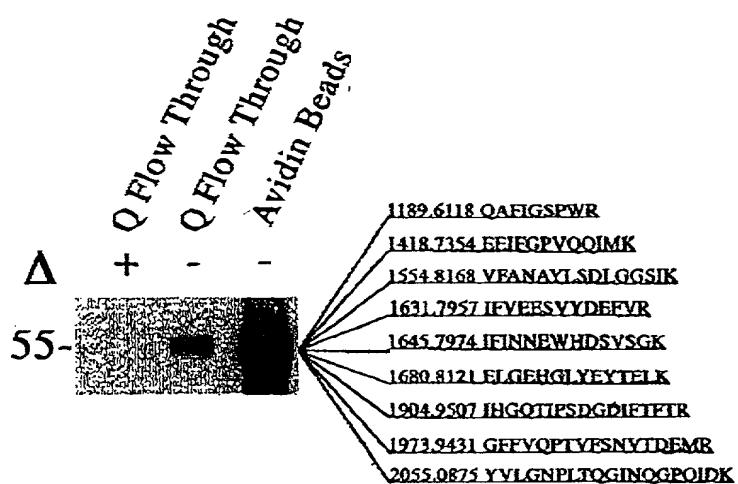
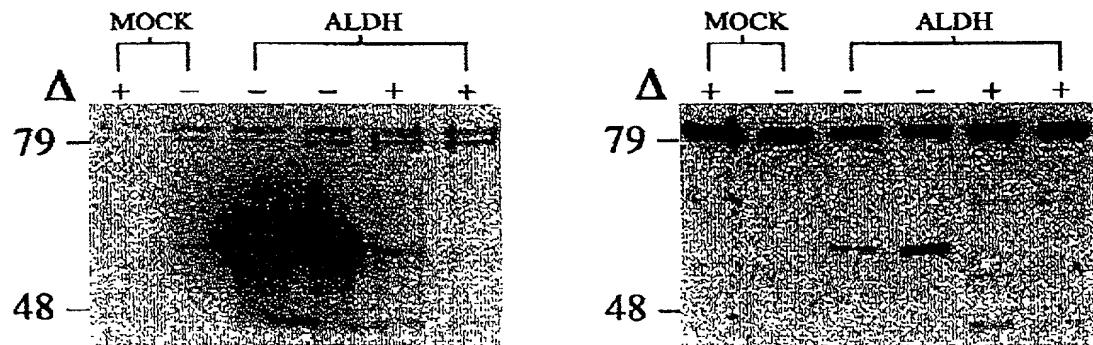


FIGURE 13

A



B



C

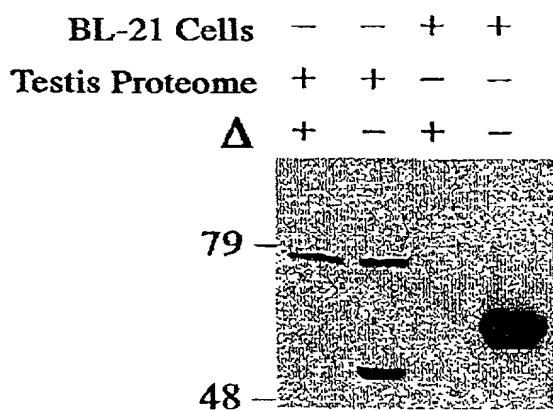
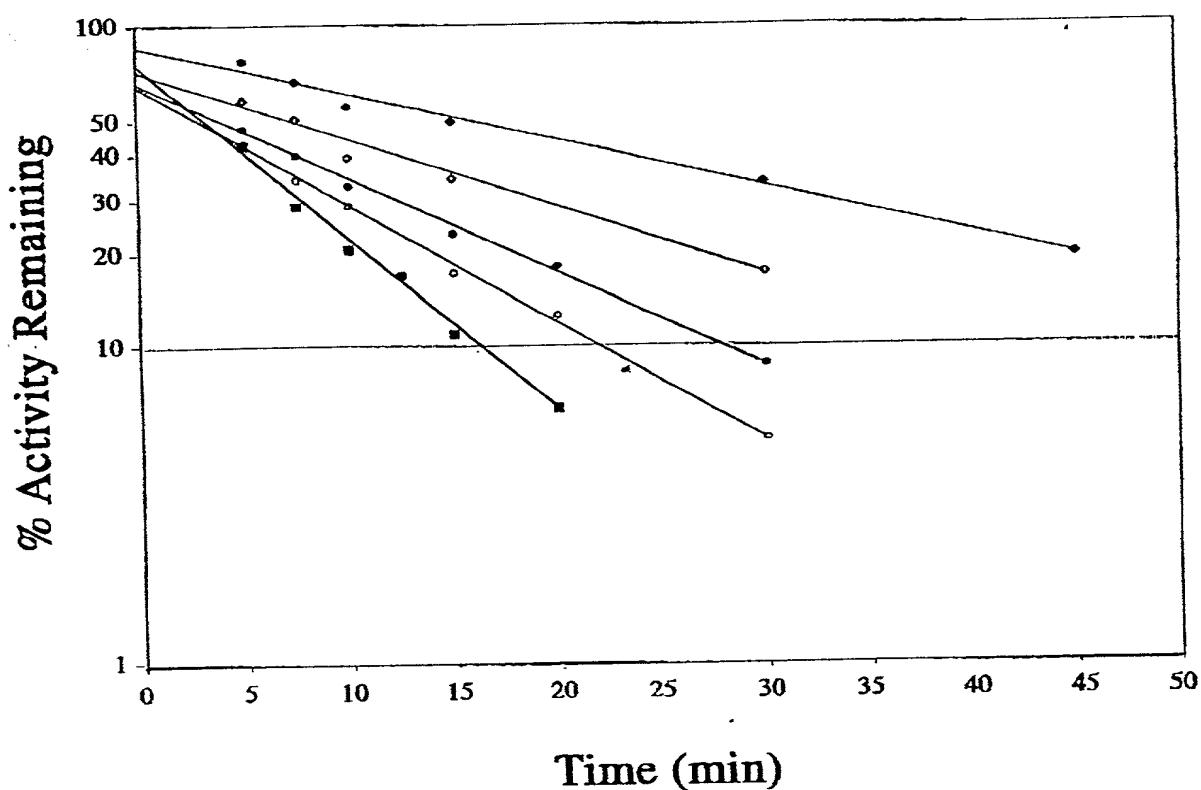


FIGURE 14

A



B

Competitor #	-	-	15	17	16	15	17	16
[Competitor ( $\mu$ M)]	0	0	5	5	5	50	50	50
$\Delta$	+	-	-	-	-	-	-	-



FIGURE 15

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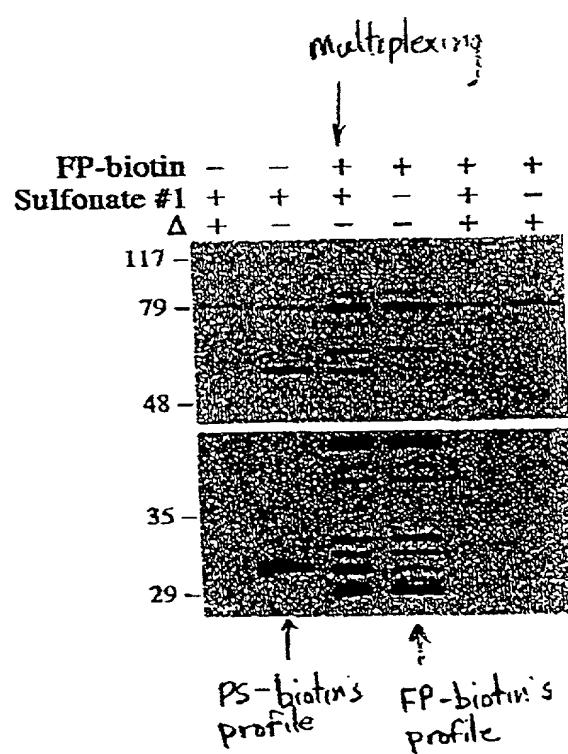


FIGURE 16

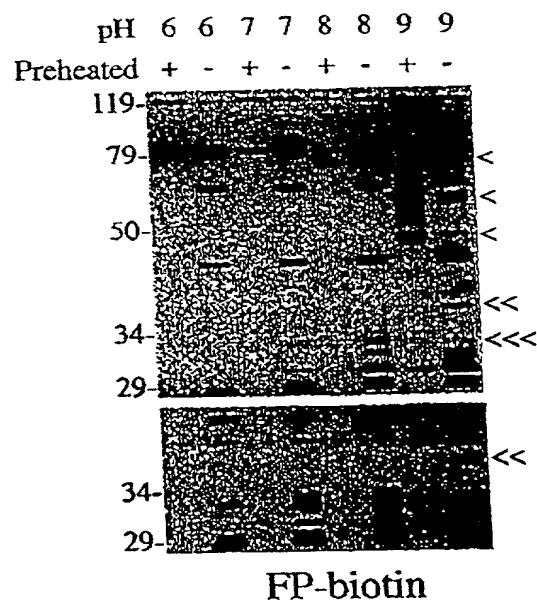
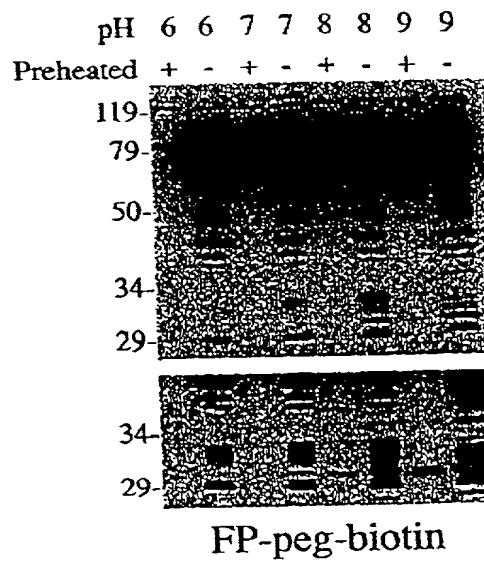


FIGURE 17

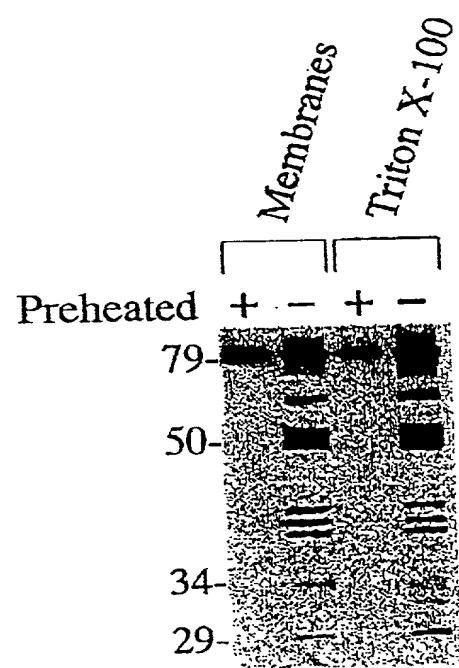


FIGURE 18

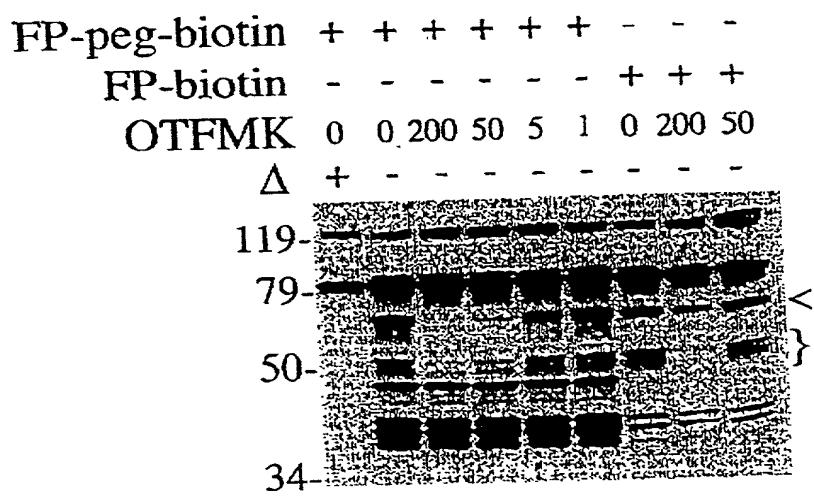


FIGURE 19

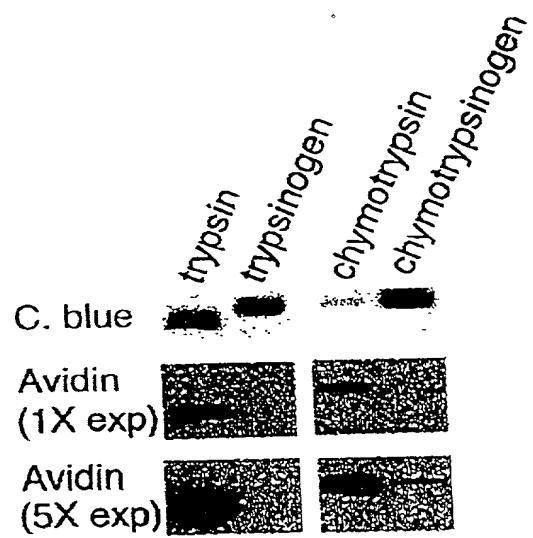
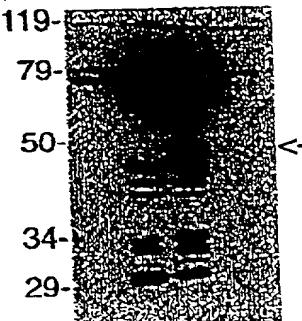


FIGURE 20

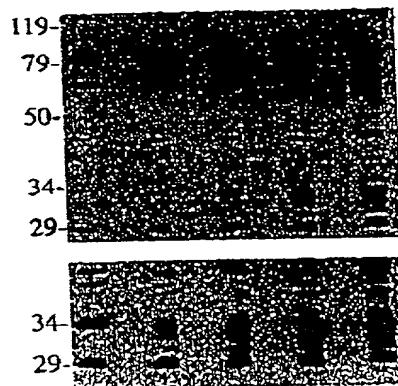
A

FP-peg-biotin - - + +  
FP-biotin + + - -  
Preheated + - - +



B

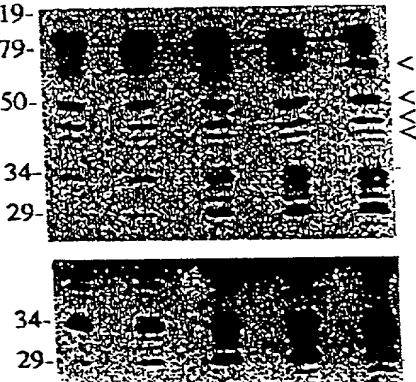
FP-biotin ( $\mu$ M) 0.5 1 1 2 2 4 4 8 8  
Preheated - + - + - + - + -



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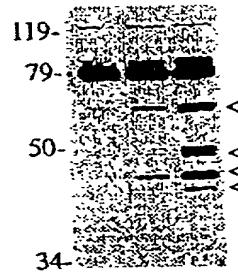
C

FP-peg-biotin ( $\mu$ M) 0.5 1 1 2 2 4 4 8 8  
Preheated - + - + - + - + -



D

FP-peg-biotin ( $\mu$ M) 1 2 8



One minute  
reaction

FIGURE 21

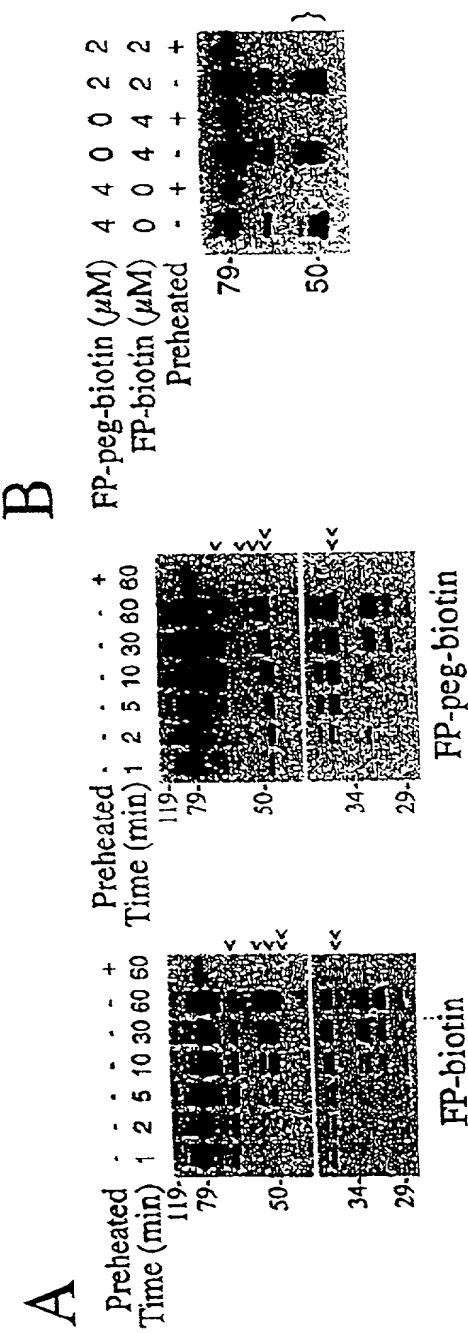


FIGURE 22

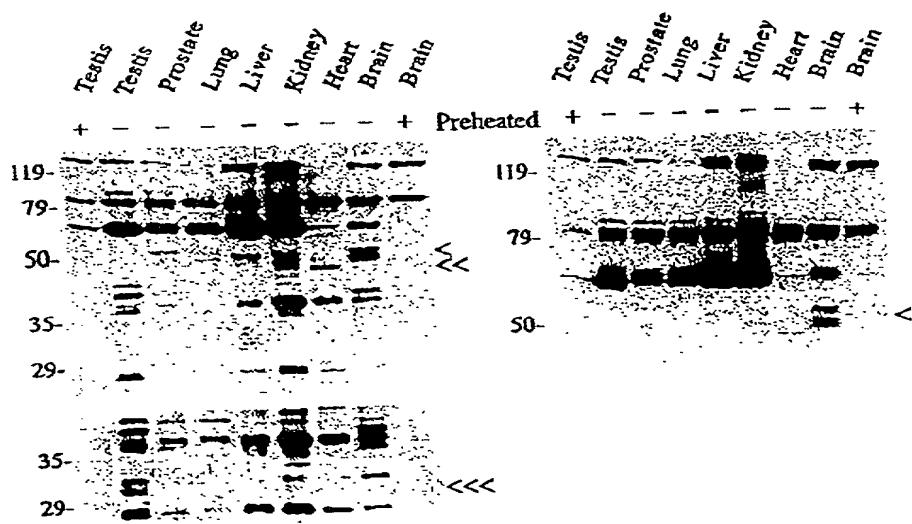


FIGURE 23

